UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/729,830	12/05/2003	Florian Von Der Mulbe	22122-00009-US	8653

23416 7590 12/23/2008
CONNOLLY BOVE LODGE & HUTZ, LLP
P O BOX 2207
WILMINGTON, DE 19899

EXAMINER	
DUNSTON, JENNIFER ANN	
ART UNIT	PAPER NUMBER
1636	
MAIL DATE	DELIVERY MODE
12/23/2008	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/729,830	VON DER MULBE ET AL.	
	Examiner	Art Unit	
	Jennifer Dunston, Ph.D.	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *14 October 2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *31-36* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *31-36* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *05 December 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☒ Other: *Exhibit I*.

DETAILED ACTION

This action is in response to the amendment, filed 10/14/2008, in which claims 1, 4, 6-23 and 29-30 were canceled, claims 31-34 were amended, and claims 35-36 were newly added. Claims 31-36 are pending.

Applicant's arguments have been thoroughly reviewed, but are not persuasive for the reasons that follow. Any rejections and objections not reiterated in this action have been withdrawn. **This action is FINAL.**

Election/Restrictions

Applicant elected Group I with traverse in the reply filed on 7/1/2005.

Currently, claims 31-36 are under consideration.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later

invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 31-36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Felgner et al (US Patent No. 5,580,859, cited in a prior action; see the entire reference) in view of Zhou et al (Human Gene Therapy, Vol. 10, pages 2719-2724, November 1999, cited as reference CZ on the IDS filed 1/28/2008; see the entire reference), Adema et al (US Patent No. 6,500,919 B1; see the entire reference); Nagata et al (Biochemical and Biophysical Research Communications, Vol. 261, pages 445-451, 1999; see the entire reference), and Fomsgaard (WO 00/29561 A2, cited in a prior action; see the entire reference). This is a new rejection, necessitated by the amendment of claim 31 to limit the tumor antigen to a human tumor antigen.

Felgner et al teach pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a modified mRNA that encodes a polypeptide, wherein the modified mRNA and wild type mRNA encode a polypeptide having an identical amino acid sequence (e.g. column 4, lines 32-45; column 5, lines 7-20; column 8, lines 28-29). Modifications taught by Felgner et al include capping the mRNA, circularizing the mRNA, or chemically blocking the 5' end of the mRNA (e.g. column 9, lines 14-27). Felgner et al teach pharmaceutical compositions comprising a modified mRNA molecule encoding a tumor antigen (e.g., column 8, lines 28-65; column 21, lines 56-67). Felgner et al teach the composition where the mRNA is designed to encode a secreted tumor antigen, which necessarily comprise a secretory leader (e.g., column 19, lines 13-16; column 20, lines 54-58). Felgner et al teach the composition is formulated for injections (e.g., column 4, lines 20-32; column 8, lines 30-32; column 9, lines 42-50; column 23,

lines 27-46). Felgner et al teach that the pharmaceutical products are for administration to a human, for example (e.g., column 5, lines 7-20).

Felgner et al do not specifically teach that the tumor antigens encoded by the modified mRNA are human tumor antigens. Felgner et al do not teach the composition where the mRNA has (i) increased GC content relative to that of a wild type mRNA encoding the polypeptide, (ii) G/C content increased at least 15% relative to that of a wild type mRNA encoding the tumor antigen, (iii) maximum GC content, and (iv) substitution of all rare codons with codons recognized by abundant cellular tRNAs.

Zhou et al teach a pharmaceutical compositions comprising mRNA encoding human gp100 melanoma-associated antigen amplified from the established melanoma cell line M12 (e.g., Title; page 2720, Plasmid Preparation; page 2720, gp100 RNA immunization and B16 cell challenge). Zhou et al teach that RNA-based cancer vaccines have some advantages over DNA, such as its safety factor and reduced potential to integrate into host chromosomes after transfection (e.g., page 2719, paragraph bridging columns). Zhou et al teach that the human melanoma-associated antigen gp100 is a melanocyte differentiation antigen recognized by HLA-A-restricted CTLs as well as antibodies in patients with melanoma (e.g., page 2719, right column). Further, Zhou et al demonstrate that mRNA encoding gp100 is capable of inducing an antibody and CTL response in mice challenged with B16 melanoma cells (e.g., page 2723). Zhou et al teach that the mRNA cancer vaccination strategy provides temporary and rapid production of protein for several days and provides an alternative approach to immunization, compared with peptides and proteins, in that it allows tumor antigens to be expressed in a normal cell background (e.g., page 2723, right column, paragraph 2).

Adema et al teach the nucleic acid sequence of SEQ ID NO: 1, which encodes the human gp100 polypeptide (e.g., column 4, lines 48-51; Example 1). Adema et al teach that it is well known in the art that the degeneracy of the genetic code permits substitution of bases in a codon resulting in another codon still coding for the same amino acid (e.g., column 4, lines 52-56; column 5, lines 1-12). Further, Adema et al teach that it is clear that for expression of a gp100 polypeptide with an amino acid sequence shown in SEQ ID NO: 2 use can be made of a derivate nucleic acid sequence with such an alternative codon composition thereby differing from the nucleotide sequence shown in SEQ ID NO: 1 (e.g., column 4, lines 52-60). Moreover, Adema et al teach that the vaccine can be composed of pure DNA, for example, a vector or virus having the DNA sequence encoding the gp100 antigen (e.g., column 10, lines 24-31). Adema et al teach that this vaccine will stimulate formation of cytotoxic T lymphocytes (e.g., column 10, lines 24-31). The codon frequencies of the coding portion of the nucleic acid sequence of SEQ ID NO: 1 are shown in Exhibit I.

Nagata et al teach that DNA immunization using the gene codon-optimized to mammals through the entire region is very effective (e.g., Abstract). Nagata et al teach that the translational efficiency of codon-substituted genes in mammalian cells is not proportional to, but does correlate with the codon adaptation index (CAI) values of the genes in mammals, although there are some exceptions, and that subsequently the polypeptide expression level in mammalian cells induces specific CTL induction levels in the mouse (e.g., page 450, right column, paragraph 3). Nagata et al state, "Taken together, our results here suggests that the polypeptide expression level becomes much higher when overall codons inserted into the expression plasmid are substituted to the optimal codons, and that DNA immunization with such a plasmid will result in

inducing much better immunological reactions as shown in Fig. 3." See page 450, right column, last full paragraph.

Fomsgaard teaches that it was known in the art that rare codons cause pausing of the ribosome, which leads to a failure in completing the nascent polypeptide chain and an uncoupling of transcription and translation (e.g., page 1, lines 30-32). Pausing of the ribosome is thought to lead to exposure of the 3' end of the mRNA to cellular ribonucleases (e.g., page 1, lines 32-33). Fomsgaard teaches that it has been shown that an exchange of the H IV codon usage to that of highly expressed mammalian genes greatly improves the expression in mammalian cell lines (e.g., page 1, lines 27-29). Fomsgaard teaches the construction of a second nucleotide sequence based on a first nucleotide sequence, where the same amino acid sequence encoded by the first and second nucleotide, and the second sequence is designed using the most frequent codons from highly expressed proteins in mammals, which are shown in Figure 1. Figure 1 shows the following:

Amino acid	One letter amino acid code	Three letter amino acid code	Codon
Alanine	A	Ala	GCC
Arginine	R	Arg	CGC
Aspargine	N	Asn	AAC
Aspartic acid	D	Asp	GAC
Cysteine	C	Cys	TGC
Glutamine	Q	Gln	CAG
Glutamic acid	E	Glu	GAG
Glycine	G	Gly	GGC
Histidine	H	His	CAC
Isoleucine	I	Ile	ATC
Leucine	L	Leu	CTG
Lysine	K	Lys	AAG
Proline	P	Pro	CCC
Phenylalanine	F	Phe	TTC
Serine	S	Ser	AGC
Threonine	T	Thr	ACC
Tyrosine	Y	Tyr	TAC
Valine	V	Val	GTG

Comparing the preferred codons to all possible codons disclosed in Figure 7 (shown below), it is clear that the preferred codon for each amino acid has maximal GC content as compared to all possible codons for the same amino acid.

aa	Σ	codons
A	Ala	GCX	GCT GCC GCG GCA
C	Cys	TGY	TGT TGC
D	Asp	GAY	GAT GAC
E	Glu	GAR	GAG GAA
F	Phe	TTY	TTT TTC
G	Gly	GGX	GGT GGC GGG GGA
H	His	CAY	CAT CAC
I	Ile	ATH	ATT ATC ATA
K	Lys	AAR	AAG AAA
L	Leu	YTX	TTG TTA CTT CTC CTG CTA
M	Met	ATG	ATG
N	Asn	AAY	AAT AAC
P	Pro	CCX	CCT CCC CCG CCA
Q	Gln	CAR	CAG CAA
R	Arg	MGX	CGT CGC CGG CGA AGG AGA
S	Ser	WSX	TCT TCC TCG TCA AGT AGC
T	Thr	ACX	ACT ACC ACG ACA
V	Val	GTX	GTT GTC GTG GTA
W	Trp	TGG	TGG
Y	Tyr	TAY	TAT TAC

Thus, when all codons are replaced with preferred codons, the coding sequence has maximal GC content while encoding the same polypeptide sequence as the starting nucleic acid sequence.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the mRNA vaccine compositions encoding a tumor antigen protein of Felgner et al to specifically encode human gp100 melanoma-associated antigen taught by Zhou et al and Adema et al, and to include codon optimization across the entire coding sequence as taught by Nagata et al and Fomsgaard, because Zhou et al teach it is within the skill of the art to make a pharmaceutical composition comprising an mRNA encoding human gp100, Adema et al teach it is within the skill of the art to modify the sequence of SEQ ID NO: 1 to encode the same gp100 protein, and Nagata et al teach it is within the ordinary skill in the art to optimize codons of a nucleic acid encoding an antigenic protein. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to replace all codons with the

preferred codons taught by Fomsgaard, because Felgner et al, Nagata et al, and Fomsgaard teach modified nucleic acid sequence. Using the known technique of codon optimization to codons recognized by abundant tRNAs in human cells would have been obvious to one of ordinary skill. Moreover, it would have been obvious to increase the G/C content of the gp100 coding sequence by at least 15% relative to that of a wild type mRNA encoding the gp100 tumor antigen, because replacement of all codons with the preferred codons taught by Fomsgaard results in a 16% increase in G/C content relative to the sequence of SEQ ID NO: 1 taught by Adema et al. This figure was obtained by comparing the codon usage shown in Exhibit I to the preferred codons of Fomsgaard and calculating the number of changes of A/T to G/C. Replacement with all rare codons with the preferred codons of Fomsgaard results in maximal G/C content, because the preferred codons have the highest C/C content possible while still coding for the same amino acid.

One would have been motivated to make such a modification in order to receive the expected benefit of increasing the expression of the antigenic protein, as taught by Nagata et al and Fomsgaard, and to provide a better immunological response as taught by Nagata et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Response to Arguments - 35 USC § 103

The rejection of claims 1, 4, 6-9, 11-16 and 29-34 under 35 U.S.C. 103(a) as being unpatentable over Felgner et al in view of Chen et al and Fomsgaard has been withdrawn in view

of Applicant's amendment to the claims in the reply filed 10/14/2008. The references do not specifically teach human tumor antigens.

The rejection of claim 10 under 35 U.S.C. 103(a) as being unpatentable over Felgner et al in view of Chen et al and Fomsgaard, further in view of Ueda et al is moot in view of Applicant's cancellation of the claims in the reply filed 10/14/2008.

Applicant's arguments filed 10/14/2008 have been fully considered as they apply to the new rejection presented above but they are not persuasive.

The response provides the following reasons why the amended claims would not have been obvious: (i) the disclosure of the primary and secondary references relate to distinct technical subject matter, and their combination as proposed would not have made the invention obvious; (ii) the state of the art as a whole did not recognize the need to enrich the GC content of an mRNA encoding a human antigen for expression in a human system; and (iii) declaration evidence provided with this response supports a finding that GC enriched mRNA encoding a human tumor antigen is more effective for anti-tumor vaccination than is the corresponding wild-type mRNA, a finding which would not have been expected in view of the complexity and unpredictability associated with generating an anti-tumor response *in vivo*. Each reason will be addressed in turn.

With respect to reason (i), the response asserts that the references cannot be properly combined to find that the subject matter would have been obvious. The response summarizes the teachings of Felgner et al, Chen et al and Fomsgaard. In footnote 1 on page 5 of the response, Applicant notes that genes from HIV are pathogenic proteins foreign to the vaccinated mice. Further, the response asserts that Felgner's showing with respect to heterologous antigens does

not provide any indication that an immune response could be generated against human tumor antigens. This argument is not found persuasive. Zhou et al and Adema et al teach that gp100 melanoma-associated antigen is an effective human tumor antigen. Zhou et al specifically demonstrate that mRNA encoding gp100 is capable of inducing an antibody response and a cytotoxic T cell response *in vivo* (e.g., page 2723). Continuing with an analysis of Felgner et al, the response characterizes the reference as "a prophetic suggestion to generate an *in vivo* T-cell immune response to a tumour antigen...and provide no disclosure which demonstrates a T-cell immune response to a tumour antigen *in vivo*, and no disclosure which demonstrates anti-tumor activity in *vivo*." This argument is not found persuasive. Compliance with the enablement requirement of 35 U.S.C. 112, first paragraph, does not turn on whether an example is disclosed. An example may be "working" or "prophetic." See MPEP 2164.02. Furthermore, if elements of an invention are well known in the art, the applicant does not have to provide a disclosure of those elements. Felgner et al rely upon the prior art for teaching specific tumor antigens. As shown by Zhou et al and Adema et al, gp100 melanoma-associated antigen is a well known human tumor antigen, which is capable of inducing anti-tumor activity *in vivo* (e.g., Zhou et al, page 2723).

The analysis of the Chen et al reference has been fully considered but is not found persuasive. The Chen et al reference has not been included in the new rejection.

The response asserts that there is no suggestion in Fomsgaard to enrich the GC content of an autologous gene. Further, the response asserts that there is no mention of a composition including an mRNA encoding a human tumor antigen, and the disclosure is not concerned with the treatment of tumors. The above rejection is based upon the combined teachings of Felgner et

al, Zhou et al, Adema et al, Nagata et al, and Fomsgaard. As set forth in the rejection above, Felgner et al teach an mRNA encoding a tumor antigen, and Zhou et al teach an mRNA encoding a human tumor antigen. Felgner et al, Zhou et al, and Adema et al teach the use of a nucleic acid encoding the tumor antigen to treat tumors. Adema et al teaches it is within the skill of the art to alter the nucleic acid sequence encoding gp100 where substitution of bases in a codon resulting in another codon still code for the same amino acid (e.g., column 4, lines 52-60). Given the teachings of Nagata et al and Fomsgaard et al directed to improved expression from codon optimized sequences to preferred codons, it would have been obvious to optimize the codons for expression in human cells, since the mRNA encodes a protein to be expressed in human cells. Applying the known technique of codon optimization would have been obvious to one of ordinary skill.

The response asserts that the most basic reason not to combine the cited references is that a skilled artisan reading Felgner et al would be provided only with a prophetic suggestion that anti-tumor efficacy might be realized upon administering a nucleic acid vaccine, but is provided with no suggestion to use a modified mRNA which is GC enriched. These arguments are not found persuasive. Compliance with the enablement requirement of 35 U.S.C. 112, first paragraph, does not turn on whether an example is disclosed. An example may be "working" or "prophetic." See MPEP 2164.02. Furthermore, if elements of an invention are well known in the art, the applicant does not have to provide a disclosure of those elements. Felgner et al rely upon the prior art for teaching specific tumor antigens. As shown by Zhou et al and Adema et al, gp100 melanoma-associated antigen is a well known tumor antigen, which is capable of inducing anti-tumor activity *in vivo* (e.g., Zhou et al, page 2723). The rejection is based upon the

combined teachings of Felgner et al, Zhou et al, Adema et al, Nagata et al, and Fomsgaard. Nagata et al and Fomsgaard teach GC enriched mRNA by codon optimization to codons recognized by abundant tRNAs. With respect to the secondary references, the response asserts that the references do not suggest modification of a nucleic acid sequence encoding an autologous antigen. Further, the response asserts that the secondary references have "no direct and apparent connection to anti-tumor vaccination," the cited references when considered cumulatively do not reasonably suggest the claimed "pharmaceutical compositions." Nagata et al and Fomsgaard teach GC enriched mRNA by codon optimization to codons recognized by abundant tRNAs. Using the known technique of codon optimization to codons recognized by abundant tRNAs in human cells would have been obvious to one of ordinary skill. A suggestion or motivation to combine references is an appropriate method for determining obviousness, however it is just one of a number of valid rationales for doing so. The Court in KSR identified several exemplary rationales to support a conclusion of obviousness which are consistent with the proper "functional approach" to the determination of obviousness as laid down in *Graham. KSR*, 550 U.S. at ___, 82 USPQ2d at 1395-97. See MPEP § 2141 and § 2143. In the instant case, it would have been within the skill of the art to improve tumor antigen mRNA in the same way as other antigenic nucleic acid sequences by optimizing the codons to preferred codons recognized by abundant cellular tRNAs as taught by Fomsgaard. One would have been motivated to make such a modification to result in increased expression of the protein as taught by Nagata et al and Fomsgaard et al, and to provide a better immunological reaction as taught by Nagata et al (e.g., page 450, right column, last paragraph).

The response asserts that the rejection is based upon improper hindsight reasoning. In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971). In the instant case, the rejection is based upon the teachings of combined teachings of Felgner et al, Zhou et al, Adema et al, Nagata et al, and Fomsgaard.

With respect to reason (ii), the response asserts that the usefulness of enriching the GC content of mRNA encoding human antigens for expression in human cells was not evident from the existing state of the art. The response notes that Chen and Fomsgaard are concerned with enhancing expression of an antigen which is heterologous to the cellular environment where expression is sought to be enhanced. This argument is not found persuasive. There is no reason that one would not have applied the technique of codon optimization to human genes to be expressed in human cells. Fomsgaard teaches preferred codons for expression in human cells. Further, Fomsgaard teaches that it was known in the art that rare codons cause pausing of the ribosome, which leads to a failure in completing the nascent polypeptide chain and an uncoupling of transcription and translation (e.g., page 1, lines 30-32). One would have been motivated to eliminate rare codons and optimize preferred codons of any sequence, even a human sequence, to improve translation in human cells.

The discussion and teachings of Robinson et al and Kim et al have been fully considered but do not overcome the rejection of record. Kim et al is cited as a "rare exception" to the "little attention" given to the possibility that expression of human proteins might be limited in human cells by the codon content of their mRNA. The response notes that there was no comparison reported between expression of human wild-type EPO as compared to human-codon optimized EPO. Further, the response asserts that Kim suggests that the promoter-proximal region should not be modified and it was found that decreasing the GC content of the promoter-proximal region resulted in improved EPO expression. The response asserts that this is a teaching away from enriching GC, at least in the promoter-proximal portions of the molecule, and at a minimum creates unpredictability as to the effects which GC content modification of nucleic acids encoding human antigens would be likely to produce. These arguments are not found persuasive. Although Kim et al teach that "decreasing the GC content of the limited region downstream of the initiator codon is advisable," (see page 299, right column, last sentence), Kim et al teach that the art clearly supports achieving high-level expression by re-engineering a coding sequence to match to the codons frequently found in human genes (e.g., page 299, right column, last paragraph). The teachings of the art would have led one to optimize the codons over the entire length of the coding sequence. Nagata et al teach that the translational efficiency of codon-substituted genes in mammalian cells is not proportional to, but does correlate with the codon adaptation index (CAI) values of the genes in mammals, although there are some exceptions, and that subsequently the polypeptide expression level in mammalian cells induces specific CTL induction levels in the mouse (e.g., page 450, right column, paragraph 3). Nagata et al state, "Taken together, our results here suggests that the polypeptide expression

level becomes much higher when overall codons inserted into the expression plasmid are substituted to the optimal codons, and that DNA immunization with such a plasmid will result in inducing much better immunological reactions as shown in Fig. 3." See page 450, right column, last full paragraph. Nagata et al teach optimizing the codons through the entire coding sequence (e.g., Abstract). Considering the evidence on the record as a whole, the prior art would not have led the ordinary skilled artisan away from optimizing the codons to codons recognized by abundant tRNAs across the entire coding sequence. Although some exceptions will exist, the art teaches that optimizing codons results in increased expression of the polypeptide in general (e.g., Nagata et al; page 450, right column, paragraph 3). Thus, the optimizing codons to produce maximal GC content would be expected to increase protein production.

With respect to reason (iii), the response notes that a second declaration of Dr. Ingmar Hoerr has been submitted. The response notes that Dr. Hoerr reports experiments which compared the activity of GC-enriched mRNAs encoding certain human tumor antigens to the activity of the corresponding wild-type mRNAs using two different assay formats which are predictive of anti-tumor utility. Specifically, the declaration shows that, in tumor challenge experiments, tumor growth was reduced more efficiently by vaccination with GC-enriched mRNA as compared with the respective wild type mRNA coding for the human tumor antigens Survivin, GP100 and TRP-2. Further experiments noted in the declaration are "ELISPOT" experiments, which showed that vaccination of animals with GC-enriched mRNA coding for the human tumor antigens MAGE-A2, MAGE-C2 and STEP led to induction of more tumor antigen specific cytotoxic T-cells than did vaccination with wild-type RNA. The response notes that this is significant since a correlation is known to exist between the generation of tumor antigen-

specific cytotoxic T-cells and the induction of immunity against tumor cells. The response asserts that these results would not have been predictable.

First, the response asserts that *in vivo* expression is not necessarily predictable from *in vitro* expression. The response cites the Robinson reference provided with the response, which states "Our experiments were based upon overexpression by transient transfection, and *in vitro* translation. The extent to which codon bias influences gene expression *in vivo* remains an open question." This argument is not found persuasive. Applicant has not provided objective evidence that *in vitro* expression does not correlate with *in vivo* expression. Even if *in vivo* expression was unpredictable, the evidence shown in the declaration is not commensurate in scope with the claims. The claims encompass any human tumor antigen, while the declaration tests Survivin, GP100, TRP-2, MAGE-A2, MAGE-C2 and STEP. If the nature of the invention is unpredictable, these results cannot be extrapolated to any human tumor antigen of any type of tumor.

Second, the response asserts that therapeutic efficacy is not predictable from *in vivo* expression. The response asserts that achieving an actual anti-tumor response *in vivo* in an animal is inherently complex and unpredictable. With respect to unpredictability, the response states the following:

> From a technical standpoint, the unpredictability of the subject matter should take into account the hurdles which must be overcome to achieve effective anti-tumor response by vaccination. For example, 1) the mRNA must enter the target cells without being digested in the extracellular space; 2) uptake of mRNA is a process which is not understood in detail. If the mRNA is incorporated by endosomes, the amount of protein which is expressed depends on the amount of mRNA which can escape from endosomes; 3) the immune response which is induced by the encoded protein depends on the cell type which uptakes the mRNA. If the cells express MHC I molecules they can induce a cytotoxic T cell response, and if they express MHC II molecules they can induce a TH1- or THD response (which

> includes a B-cell response); 4) tumor antigens are self-antigens, and it is necessary to overcome the tolerance of the immune system, which normally does not recognize self-antigens; 5) induction of an immune response does not alone ensure that the immune system is able to inhibit tumor growth. Although it is known that a cytotoxic T cell response plays an important role, the responsible mechanisms are not fully elucidated.

The response asserts that due to this unpredictability, the improved anti-tumor response *in vivo* achieved using GC enriched mRNA coding for tumor antigens was unexpected, even assuming that improved *in vitro* expression could have been expected (which Applicant does not concede).

These arguments are not found persuasive. Applicant is essentially stating that immunization by administration of an mRNA vaccine is an unpredictable venture, regardless of the GC content of the message. The response points to difficulties with delivery of the nucleic acid such that protein is produced, tolerance of the immune system, which normally does not recognize self-antigens, and induction of the immune response does not alone ensure that the immune system is able to inhibit tumor growth. Applicants arguments presented at pages 4-8 in the reply filed 1/28/2008 speak to the predictability of the art and the requirement for routine experimentation to optimize a particular, specific vaccine embodiment. Furthermore, these arguments are not persuasive in overcoming the above rejection, because Adema et al teach that gp100 is a melanoma-associated tumor antigen that can be used in vaccines (e.g., Abstract). Further, Adema et al teach that the vaccine can be composed of pure DNA, for example, a vector or virus having the DNA sequence encoding the gp100 antigen (e.g., column 10, lines 24-31). Adema et al teach that this vaccine will stimulate formation of cytotoxic T lymphocytes (e.g., column 10, lines 24-31). Moreover, Adema et al teach it is within the skill of the art to alter the nucleic coding sequence of gp100 to substitute one codon for another when the codons code for

the same amino acid (e.g., column 4, lines 48-60). Zhou et al provide experimental data demonstrating that an mRNA encoding gp100 is capable of inducing both an antibody response and cytotoxic T cell response in BALB/C mice (e.g., page 2723). Zhou et al teach it is within the skill of the art to overcome difficulties with gene delivery by using a delivery vehicle such as HVJ-liposomes (e.g., paragraph bridging pages 2722—723). As noted in the declaration, the murine B16 melanoma tumor model which makes use of B16 melanoma cells isolated from C57BL/6 mice provides a syngenic tumor model for evaluating the efficacy of anti-tumor vaccines. Dr. Hoerr states at paragraph 8 of the declaration filed 11/14/2008, "Since there is no *in vivo* human model available for use by workers in this field, this model is an important and art-recognized model for evaluating human tumour vaccination strategies." Zhou et al evaluate the efficacy of the mRNA vaccine encoding gp100 in C57BL/6 mice challenged with B16 melanoma cells (e.g., page 2720, Mouse strain and cell culture; page 2720, gp100 RNA immunization and B16 cell challenge). Thus, Zhou et al use an art-accepted model to test the efficacy of an mRNA vaccine encoding gp100. It would have been predictable for one to use the mRNA encoding gp100 as a pharmaceutical. Given the teachings of Nagata et al and Fomsgaard directed to improved expression of nucleic acid sequences comprising optimized codons recognized by abundant tRNAs, one would expect an improved response due to increased expression. Nagata et al state, "Taken together, our results here suggests that the polypeptide expression level becomes much higher when overall codons inserted into the expression plasmid are substituted to the optimal codons and that DNA immunization with such a plasmid will result in inducing much better immunological reactions as shown in Fig. 3." See page 450, right column, last full paragraph. Since DNA is transcribed to mRNA, and the mRNA is what is

recognized by the tRNAs and translated to protein (e.g., Fomsgaard, page 1, lines 30-33), one would expect the use of optimal codons, which necessarily result in maximal GC content, to result in higher expression and better immunological reactions. Thus, one would have expected mRNA compositions encoding gp100 to be active as a vaccine *in vivo* and would have expected maximizing the GC content by optimizing the codons to codons recognized by abundant tRNAs to result in an improved immune response.

The response notes that Felgner et al do not teach human tumor antigens. It is noted that Felgner et al teach tumor antigens, and Zhou et al and Adema et al teach the gp100 human tumor antigen.

Based on the evidence as a whole, the claimed pharmaceutical compositions would have been obvious for the reasons set forth above.

Response to Amendment -- Declaration of Dr. Hoerr

The declaration under 37 CFR 1.132 filed 11/14/2008 is insufficient to overcome the rejection of claims 31-36 based upon the Felgner et al, Zhou et al, Adema et al, Nagata et al, and Fomsgaard references applied under 35 U.S.C. 103(a) as set forth above.

The declaration provides evidence of the following:

a) In tumor challenge experiments, it was demonstrated that tumor growth is reduced more efficiently by vaccination with GC-enriched mRNA, as compared with the corresponding wild type mRNA coding for the human tumor antigens Survivin, GP100 and TRP-2.

b) ELISPOT experiments showed that vaccination with GC-enriched mRNA coding for the tumor antigens MAGE-A2, MAGE-C2 and STEP leads to the induction of more tumor

antigen-specific cytotoxic T cells than does vaccination with the corresponding wild type mRNA. The declaration notes that these results are significant, since it has been shown in recent years that induction of tumor antigen-specific cytotoxic T cells is necessary for induction of immunity against tumor cells *in vivo*.

The declaration attempts to set forth evidence of unexpected results when using a GC-enriched mRNA as compared to a wild-type mRNA. However, considering the evidence on the record as a whole, improved anti-tumor response with a GC-enriched mRNA as compared to a wild type mRNA would have been expected.

Adema et al teach that gp100 is a melanoma-associated tumor antigen that can be used in vaccines (e.g., Abstract). Further, Adema et al teach that the vaccine can be composed of pure DNA, for example, a vector or virus having the DNA sequence encoding the gp100 antigen (e.g., column 10, lines 24-31). Adema et al teach that this vaccine will stimulate formation of cytotoxic T lymphocytes (e.g., column 10, lines 24-31). Moreover, Adema et al teach it is within the skill of the art to alter the nucleic coding sequence of gp100 to substitute one codon for another when the codons code for the same amino acid (e.g., column 4, lines 48-60). Zhou et al provide experimental data demonstrating that an mRNA encoding gp100 is capable of inducing both an antibody response and cytotoxic T cell response in BALB/C mice challenged with B16 melanoma cells (e.g., page 2723). As noted in the declaration, the murine B16 melanoma tumor model which makes use of B16 melanoma cells isolated from C57BL/6 mice provides a syngenic tumor model for evaluating the efficacy of anti-tumor vaccines. Dr. Hoerr states at paragraph 8 of the declaration filed 11/14/2008, "Since there is no *in vivo* human model available for use by workers in this field, this model is an important and art-recognized model for evaluating human

tumour vaccination strategies." Zhou et al evaluate the efficacy of the mRNA vaccine encoding gp100 in C57BL/6 mice challenged with B16 melanoma cells (e.g., page 2720, Mouse strain and cell culture; page 2720, gp100 RNA immunization and B16 cell challenge). Thus, Zhou et al use an art-accepted model to test the efficacy of an mRNA vaccine encoding gp100. It would have been predictable for one to use the mRNA encoding gp100 as a pharmaceutical. Given the teachings of Nagata et al and Fomsgaard directed to improved expression of nucleic acid sequences comprising optimized codons recognized by abundant tRNAs, one would expect an improved response due to increased expression. Nagata et al state, "Taken together, our results here suggests that the polypeptide expression level becomes much higher when overall codons inserted into the expression plasmid are substituted to the optimal codons and that DNA immunization with such a plasmid will result in inducing much better immunological reactions as shown in Fig. 3." See page 450, right column, last full paragraph. Since DNA is transcribed to mRNA, and the mRNA is what is recognized by the tRNAs and translated to protein (e.g., Fomsgaard, page 1, lines 30-33), one would expect the use of optimal codons, which necessarily result in maximal GC content, to result in higher expression and better immunological reactions. Thus, one would have expected mRNA compositions encoding gp100, or another known human tumor antigen, to be active as a vaccine *in vivo* and would have expected maximizing the GC content by optimizing the codons to codons recognized by abundant tRNAs to result in an improved immune response.

Conclusion

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Low can be reached at 571-272-0951. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR

system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Jennifer Dunston, Ph.D.
Examiner
Art Unit 1636

/JD/

/Celine X Qian /
Primary Examiner, Art Unit 1636